Synopsis

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Title of Thesis: "Design and Evaluation of Baricitinib-Loaded Niosomal Drug Delivery System for Rheumatoid Arthritis"

Abstract of work done

The study aimed to design and optimize Baricitinib-loaded biomimetic (BCTB) niosomes for treating complete Freund's adjuvant (CFA)-induced rheumatoid arthritis (RA) in a rat model mimicking human joint inflammation. To enhance therapeutic efficacy and reduce infection risks, Human-Serum-Albumin (HSA)-decorated BCTB-loaded niosomes were formulated and evaluated against marketed BCTB tablets. Niosomes were developed using sonication followed by the reverse-phase evaporation method and optimized via Box-Benken-Design. They were characterized for vesicle size, zeta-potential, entrapment efficiency, morphology, in-vitro release, and molecular interactions (FTIR, DSC, XRD). In-vivo evaluation was conducted on Wistar rats, assessing interleukin-6 levels, body weight changes, arthritic index, radiographic findings, hematological parameters, and histopathology of rear paw joints. Optimized niosomes exhibited a spherical morphology with a vesicle size of 161.7±0.1nm, PDI 0.145±0.013, zeta-potential -52±7.68mV, and high entrapment efficiency (93.40±1.8%). FTIR, DSC, and XRD confirmed no significant drug-excipient interactions. HSA-decoration enhanced therapeutic effects of BCTB by prolonging drug release, mitigating adverse effects, and improving disease outcomes. BCTB niosomes significantly alleviated clinical and histopathological signs of RA, showing comparable efficacy to marketed BCTB tablets in the 2mg/kg treated group. These findings highlight the potential of BCTB-loaded niosomes as a targeted drug delivery platform for RA treatment, offering improved bioavailability, sustained release, and reduced systemic toxicity.

Objective:

The main objective of this research study are as follows:

- To develop and evaluate HBN (Human Serum Albumin based baricitinib loaded niosomes) for the management of Rheumatoid Arthritis.

- In-vitro release assay of HBN as performance test.
- Pharmacokinetic studies of optimized HBN nanoformulation.
- To assess in-vivo anti-arthritic activity of developed HBN formulation against CFA (Complete freunds adjuvant) induced immunological RA.

Introduction:

Baricitinib (BCTB), is a small molecule inhibitor of Janus kinase (JAK) 1 and 2. It is a medication used primarily to treat rheumatoid arthritis and severe COVID-19. While it can be effective, it has several limitations and potential side effects; it suppresses the immune system, which can increase the risk of infections, including serious infections like tuberculosis, fungal infections, and bacterial infections. There is an increased risk of blood clots, including deep vein thrombosis (DVT) and pulmonary embolism (PE), especially in patients with a history of these conditions. It can also cause increases in total cholesterol, LDL cholesterol, and HDL cholesterol levels. There is a potential increased risk of developing certain types of cancers due to the immunosuppressive nature of the drug, though this risk needs more long-term study. Delivering BCTB through a ligand-linked novel drug delivery system has the potential to mitigate some adverse effects, but it is unlikely to eliminate all risks associated with the drug. The primary goal of innovative drug delivery systems is to improve the efficacy and safety profile of medications by optimizing how the drug is released, distributed, and targeted within the body. By directing BCTB specifically to the affected tissues or cells, such as inflamed joints in rheumatoid arthritis, the drug's systemic exposure can be minimized, reducing the risk of systemic side effects like immunosuppression and blood clots. Enhanced delivery mechanisms may allow for lower dosages of BCTB while maintaining its therapeutic efficacy, thereby reducing the likelihood of side effects associated with high drug concentrations.

Niosomes are non-ionic surfactant-based nano-vesicles that were designed as an alternative controlled drug delivery technology to liposomes to solve sterilization, large-scale manufacturing, and stability issues. These systems can serve as drug reservoirs and deliver active substances in a controlled way. Compared with other micro and nano-encapsulation technologies, niosomes offer several benefits, including high stability, affordable cost, ease of formulation, and scaling-up. Additionally, HSA has also been reported to selectively disperse in RA-inflamed joints, suggesting that it might be a promising carrier for site-specific delivery to inflamed joints.

Methodology and Results:

The proposed work is expected to resolve the problems associated with the baricitinib drug by designing a biomimetic nano-carrier. But for the formulation and development of any advance drug delivery system basic analytical procedures like UV spectroscopy acts as pillar in estimation

of drug. The researchers have urge for a precise and very sensitive analytical technique. Such techniques should be easy, rapid, accurate, precise as well as stable which drives the product development process earlier and to greater extent of success. Hence, any of the drug molecules needs a suitable analytical method for characterization as an important step of preformulation studies in designing a drug delivery system. Preformulation is a crucial stage within the research and development process, whereby the physical, chemical, and mechanical attributes of a pharmacological ingredient are thoroughly examined and characterised. The primary objective of this phase is to facilitate the creation of a stable, safe, and efficacious dosage form. The initial stage of the discovery process facilitates the identification of a therapeutic molecule that will proceed to the development phase. A comprehensive comprehension of the physical, chemical, and mechanical characteristics of a medicine eventually serves as a basis for the design of its formulation or provides justification for molecular modifications, or simply verifies the absence of substantial obstacles to the compound's progress. BCTB was found to be white to off-white colored powder with no characteristic odor. The UV spectra obtained from validated method had its λmax peak at 249.4 nm in phosphate buffer pH 7.4. FTIR spectrum of BCTB and mixture of excipients was obtained using FTIR spectrophotometer by spread as a thin layer on potassium bromide cell and then scanned between 4000 - 500 cm -1. The result shows that the peaks of BCTB and Physical Mixture have similar peaks which means it does not have interaction with excipients. The Point in DSC graph of BCTB indicates the endothermic peak, indicating significant crystalline structure which rearranges on heating to a amorphous structure before melting at about 218°C. The human serum based baricitinib loaded niosomes (HBN) were prepared in different batches in which concentrations of span 60 (Sp 60) and cholesterol (CH) were varied. The preparation method aids to synthesize nano-vesicles in a size range of 159.6-264.5 nm, having a zeta potential (ZP) range between -13 to -52. The average size range of nano-vesicles varied from 159.6-264.5 nm, which was influenced by changing the concentration of Sp 60, CH, and Sonication time. Upon analyzing the data statistically, the model proposed (quadratic) was significant with a value of $p< .0001$, F-value (2039.31), and a coefficient of correlation i.e., $R2= 0.999$ respectively. ZP range was found to be in limits of -13 to -52 and ANOVA was performed to show the factors. The p-value (< 0.0001) less than 0.05 denotes the model terms as significant. The negative coefficient of ZP may be due to the dipole orientation of the ethoxy group of Sp 60 (Shahraeini et al., 2020). Moreover, the use of Dicetyl phosphate (charge inducer) synergizes the negative charge on the vesicle surface (Aparajay & Dev, 2022). The higher ZP negative values indicated that vesicles were more strongly repel one another and less likely to

flocculate. The best-fitted model was quadratic for ZP where R2= 0.966. EE of HBN was found to be in a range of 57.86-94.26%. Adjusted and predicted values of R2 obtained were 0.989 and 0.977 respectively. The negative coefficient of Sp 60 indicated a reduction in % EE with enhancement in Sp 60 concentration, and the positive coefficient of CH showed an increase in % EE with an upsurge amount of CH. It has been previously identified that CH enhances the bilayer hydrophobicity and stability and declines the vesicle permeability which leads to efficient trapping of the hydrophobic drug into bilayers up to a limit after that increasing the CH levels, % EE reduces because a higher amount of CH may compete with the drug for packing space. The optimized HBN formulation FTIR spectra did not exhibit the expected characteristic bands of raw BCTB, but they showed functional groups specific to Sp 60 with a minor shift. These observations suggest the successful entrapment of BCTB inside the niosomal nano-vesicles. The characteristic peak of BCTB was not detected in the HBN DSC thermogram, only the Sp 60 peak was observed. This suggests complete encapsulation of BCTB within HBN nano-vesicles. DSC results were further confirmed by XRD analysis to figure out whether it is crystalline or amorphous. The pure BCTB spectra exhibited several strong peaks at 12.52º 2θ, 14.04º 2θ, 15.41º 2θ, 16.6º 2θ, 19.14º 2θ and 25 .39º 2θ, which suggested its crystalline behaviour. The physical mixture spectra (PHM) demonstrated that BCTB kept its crystallinity during physical mixing since they displayed a less intense peak than pure BCTB. The lyophilized powder of HBN did not exhibit any sharp peak, which revealed that BCTB is entrapped within the matrix of niosomes and acts as an amorphous solid dispersion in nature. The surface morphology of optimized HBN was represented with the help of an TEM image. Optimized nano-vesicles were seeming to be nearly spherical with a smooth surface and dispersed evenly with minimal aggregation. The TEM picture clearly shows that most of the niosomal vesicles in the optimized formulation were less than 180 nm in size and spherical. The market formulation (Barinat 4) showed instantaneous release profile of 98.522±0.421%, while the rate of release for 2 mg and 4 mg HBN was 51.527± 0.808, and 44.884±0.681% respectively in 12 hrs. In the initial phase rapid release of BCTB was observed in HBN formulations followed by sustained release in the next 48 hours. The drug's prolonged release after 12 hours was caused by the sluggish diffusion of BCTB from niosomal vesicles. In the treatment of chronic arthritic conditions, sustained release of BCTB may assist in reducing the frequency of oral administration and dose-related adverse effects. The release kinetics of optimized HBN formulation were investigated and found to follow Takeru Higuchi's equation. It depicts the release of BCTB from HBN formulations as the square root of a time and diffusion-dependent controlled release process. Which is further well conformed by the release exponent

(n>1) of the Korsmeyer-Peppas power law model. It specifies the type of diffusion by n value, which is larger than 0.89, suggesting that the drug release from the system follows super case II transport. This type of transport includes state transition and erosion of polymer with diffusion (Unagolla & Jayasuriya, 2018). Barinat 4 showed the Korsmeyer-Peppas model, which exhibited that the release of BCTB by dissolution and diffusion of the polymer matrix (Singhvi & Singh, 2011). Furthermore, HBN was also found to be stable at different pH, temp., and bile salt conc. The in-vivo results of % Inhibition in Paw edema showed that rats treated with 2mg HBN, 4mg HBN and 4mg MKT tablets exhibited a reduction in edema volume of 77.96±1.95 %, 79±0.81 %, and 73.30±0.8 % respectively in comparison to the DC group. When compared to the DC treatment group, rats treated with 2 mg HBN, 4 mg HBN, and MKT formulation showed a progressive increase in body weight of 2.2%, 2.1%, and 1.5%, respectively. Rats in the DC group, however, lost weight significantly (-3.7%) compared to those in the NC group. The arthritic score for right hind paws of different treatment groups was recorded at 2, 5, 10, and 14 days after CFA injection by assessing paw thickness, ankle diameter, and visual examination of swelling and redness. When compared to the DC group, other treatment groups showed a substantial decline in the arthritic index ($p<0.005$). In comparison to the MKT tablet group, 2 and 4 mg HBN groups exhibited improved arthritic index, indicating fewer symptoms of inflammation and arthritis. In the current experimental study, we observed a significant ($*P<0.05$) increase in HGB and RBC levels and a reduction in ESR and R-Factor value in HBN and MKT Tablet groups, when compared to the DC group. The results imply that the HBN formulations altered the hematological state to a level that was very near to normal and confirmed their anti-arthritic activity. IL-6 serum level was also quantified by sandwich ELISA technique on 0, 5, 10, and 14 days of treatment and it was found that IL-6 levels of the DC group rat's serum were significantly greater ($p<0.0001$) in comparison to the NC group. However, rats treated with 2 HBN and 4 HBN showed considerably lower levels ($p<0.0001$) than the DC group and the MKT tablet group exhibited a decrease in IL-6 conc. than NC group. The obtained results suggested that the developed HBN formulation decreased only elevated IL-6 levels and did not contribute to viral infections associated with low levels of IL-6. Each treatment group underwent radiographic evaluation on day 14 following the CFA injection. When comparing X-ray images of rats with induced arthritis (DC group) to normal rat groups (NC group), the inflammation of the synovial membrane and cartilage destruction with narrowing of the space between the bones were observed in the DC group. In contrast, the HBN and MKT tablet formulations treated rat's ankle joints showed only modest erosions in these regions. The therapeutic efficacy of HBN formulations was further estimated by inflammation and

tissue damage assessment in bone and cartilage via H&E staining. HBN formulations and MKT Tablet groups exhibited intermediate inflammation with no signs of bone and cartilage erosion. In-vivo toxicity study of HBN was performed histological analysis of the major organs such as the liver, kidney, and spleen of Wistar albino rats. The observations corroborated the non-toxic and biocompatible nature of the HBN formulations.

Novel Gradient Flow HPLC system was developed for Pharmacokinetic evaluation of developed formulation. After oral administration of treatment, blood and knee joint samples were withdrawn from each treatment rat group. The serum was isolated using cooling centrifuge, and BCTB was isolated from serum by liquid-liquid isolation technique and analysis of drug was done by a developed HPLC method. The different pharmacokinetic parameters in 2mg/kg dose HBN group, 4 mg/kg HBN group, and the marketed barinat tablet (4 mg/kg) group were detected by using PK Solver software. The observed results of the pharmacokinetic study showed that prepared HBNs deliver a large amount of drug payload to the target site with a sustained release mechanism in the animal (rat model) knee joint.

Conclusions: The current research work on HBN discovered successful preparation along with enhanced in-vivo therapeutic activity. We aimed to concentrate on a unique technique for the treatment of RA by sustaining the release of BCTB and targeting the drug to inflamed joints with the help of human serum albumin. Various process and formulation variables were assessed by which an optimized niosomal formulation was prepared. The formulation was optimized by adequate particle size, low polydispersity index, high entrapment efficiency, desired release profile, and therapeutic efficacy with low toxicity. The results of serum biochemical analysis and histopathological assessment proved that HBN in niosomal form showed desired in-vivo results compared to conventional dosage form against CFA-induced RA.

The development of sustained-release, targeted niosomes of BCTB presents a promising nano-therapeutic strategy for the effective management of rheumatoid arthritis (RA). By incorporating BCTB into niosomal carriers, the formulation successfully enhances drug stability, bioavailability, and controlled release, reducing the need for frequent dosing and improving patient compliance. The targeted delivery to inflamed joints minimizes systemic side effects and enhances therapeutic efficacy, overcoming limitations associated with conventional oral and injectable treatments.

In-vitro and *in-vivo* studies confirm that HBN exhibits prolonged circulation, improved drug retention in the inflamed synovium, and enhanced anti-inflammatory effects. This novel formulation holds significant potential for clinical translation, offering a safer, more effective, and patient-friendly alternative for RA treatment. Further long-term pharmacokinetic and clinical studies will be

crucial in optimizing the formulation and establishing its superiority over existing RA therapies.

Impact of the Work and possible Future Work

The present work has successfully established the in vivo pharmacokinetic, pharmacodynamic, and biodistribution profile of baricitinib-loaded niosomes, demonstrating enhanced systemic exposure, preferential accumulation in inflamed joint tissues, and sustained therapeutic activity in validated rheumatoid arthritis models. These findings strongly support the translational potential of the developed niosomal platform. Furthermore, the incorporation of active targeting strategies through surface functionalization with disease-relevant ligands has effectively enhanced selective uptake by inflamed synovial tissues and immune cells, thereby improving therapeutic precision while minimizing off-target distribution.

Despite promising outcomes, further investigations are warranted to advance the clinical translation of the baricitinib-loaded niosomal drug delivery system for the treatment of rheumatoid arthritis. Building upon these advances, future investigations should focus on optimizing ligand density and spatial orientation, evaluating dual-targeting or stimuli-responsive niosomal systems, and exploring personalized targeting approaches based on disease severity and biomarker expression. Such refinements may further enhance targeting specificity, therapeutic durability, and clinical outcomes in rheumatoid arthritis management.

To assess the chronic nature of rheumatoid arthritis therapy, long-term safety, immunogenicity, and toxicity assessments following repeated administration are essential to establish the clinical viability of niosomal baricitinib. These evaluations should encompass systemic toxicity, hematological compatibility, and immune modulation profiles. To facilitate industrial translation, future efforts should focus on process optimization, scale-up feasibility, and manufacturing robustness, including evaluation of batch-to-batch reproducibility and regulatory compliance.

Exploration of alternative and patient-centric routes of administration, including transdermal, intra-articular, or topical delivery, may provide localized therapeutic benefits, improved patient adherence, and reduced systemic adverse effects, particularly in long-term rheumatoid arthritis management.

Finally, mechanistic studies addressing cellular uptake pathways, intracellular trafficking, and drug release kinetics within synovial fibroblasts, macrophages, and immune cells will be pivotal in refining formulation design and predicting clinical performance.

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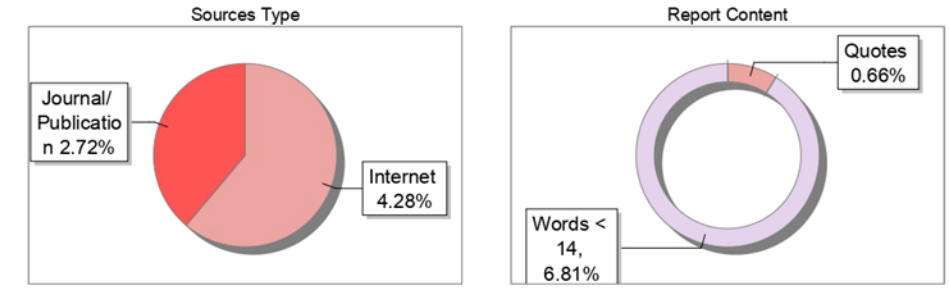

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Publication:

1. **Rani Laxmi,** Mathur Pooja, Verma Ravinder, Kumar Vivek, Mishra AK, Sahoo PK. Translation Research in Therapeutic Approaches from Conventional to Novel Nano-therapeutics for Rheumatoid Arthritis Treatment. Current Rheumatology Review. 2024 April 15; 21(1):37-53.
2. **Rani Laxmi,** Mishra AK**,** Neha SL, Dewangan HK, Sahoo PK. An Estimation of Baricitinib by AQbD-driven UV Spectrophotometry Development and Validation Process. Letters in Drug Design & Discovery. 2024 Nov 1; 21(14):2958-2966.
3. **UK Design patent** for "Instrument to Evaluate the Stability of Pharmaceutical Products" was granted on 12 Dec. 2023.

4. Mishra AK, Neha SL, **Rani Laxmi,** Dewangan HK, Sahoo PK. QbD assisted development and validation of UV spectroscopic method in estimation of silymarin. Letters in Drug Design & Discovery. 2024 Apr 1; 21(5):888-96.
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6. Paroha Shweta, Verma Juhi, Singh Chandel, **Rani Laxmi**, et al, Augmented Therapeutic Efficacy of Gemcitabine Conjugated Self-Assembled Nanoparticles for Cancer Chemotherapy, Journal of Drug Delivery Science and Technology, Elsevier, volume 76, October 2022. Impact factor- 5.06
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10. Mishra AK**, Rani Laxmi**, Singh R, Dewangan HK, Sahoo PK, Kumar V. Nanoinformatics and nanotechnology in anti-inflammatory therapy: A review. Journal of Drug Delivery Science and Technology. 2024 Feb 1:105446.
11. Nanoparticles for Targeted Drug Delivery Systems with Cancer Therapy in Perspective. Accepted for publication in the book Springer Nature (Book: Drugs, from bench to bedside: A

methodological compendium).

Conference/workshop/seminars:

Oral presentations

Rani, L. gave an oral presentation on the topic "Development and Evaluation of Human Serum Albumin Based Niosomes Nanocarrier for Therapy against Rheumatoid Arthritis" 2022 (22nd to 23rd August) at G D Goenka University Gurugram, India and awarded best paper.

Rani, L. gave an oral presentation on the topic "Development of Ligand-based drug delivery system for management of Rheumatoid Arthritis"2022 AT Institute of Nano Medical Sciences, University of Delhi.

Rani, L. participated and successfully completed one week UGC networking training course under Module B-1 on "Targeting New Echelons in Drug Discovery and Analysis" from 27 January to 01 February 2020 at University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh.

Lakshmi

Signature of Student

Signature of Supervisor